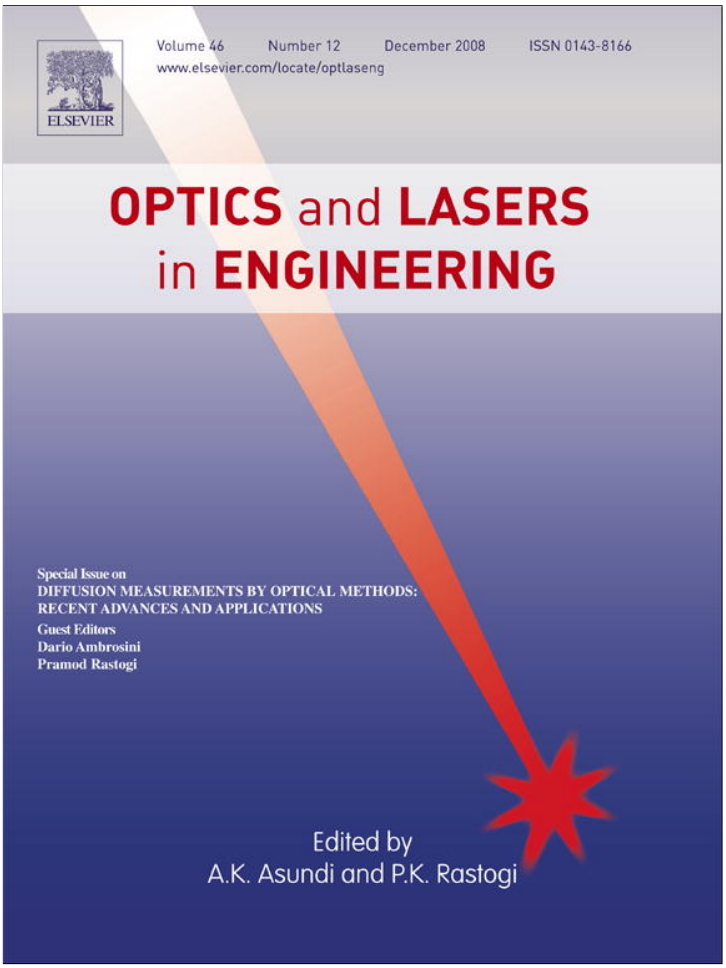

Optics and Lasers in Engineering 46 (2008) 915–920

Contents lists available at ScienceDirect

Optics and Lasers in Engineering

journal homepage: www.elsevier.com/locate/optlaseng

Measurement of *Retinalamin* diffusion coefficient in human sclera by optical spectroscopy

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ARTICLE INFO

Available online 2 September 2008

Keywords:
Drug delivery
Retinalamin
Sclera
Diffusion coefficient
Optical spectroscopy

ABSTRACT

The use of cytomedines (such as *Retinalamin*) in clinical practice has shown high effectiveness of the medicaments in ophthalmology. The study of diffusion of *Retinalamin* in scleral tissue is important for estimation of a drug dose delivered into inner tissue of eye, time of drug action, etc. *In vitro* measurements of spectral reflectance of sclera interacting with aqueous solution of *Retinalamin* have been carried out. Ten human sclera samples were included in the study. The results of the experiments have shown that penetration of *Retinalamin* into scleral tissue leads to the decrease of scleral reflectance due to optical immersion. Estimation of diffusion coefficient of studied solution has been made on the basis of analysis of optical reflectance dynamics of the sclera samples. The diffusion coefficient of *Retinalamin* in human scleral tissue was evaluated as $(1.82 \pm 0.14) \times 10^{-6}\,\mathrm{cm}^2/\mathrm{s}$. The results are important for treatment of partial optic atrophy observed at primary open-angle glaucoma and others eye diseases.

1. Introduction

Metabolic disorder in optic nerve at its partial atrophy is developed as a result of inflammation, intoxication, blood circulation disorder, glaucomatous optic neuropathy, and others [1]. The problem of its correction is an actual one. Many years' experience of the use of cytamins (such as *Retinalamin*) in clinical practice has shown high effectiveness of the medicaments in ophthalmology [1–5]. Cytamins are a large group of remedies that have the ability to control the most important biological processes in the cells of human body [6]. *Retinalamin* consists of calf's or pig's retina polypeptides Ala-Glu-Asp-Gly (molecular mass 10 kDa). It regulates metabolic processes in retina, stimulates functions of cell elements, promotes improvement of functional interaction of pigment epithelium and external segments of photoreceptors, and increases retinal macrophage activity [1–3].

Eye tissues, especially those that are responsible for visual functions, are well isolated from systemic blood circulation by a multitude of barriers. The barriers provide for a high level of soluble selection. At the system application of drug only 0.01–0.07% of injected dose reaches the eye tissues [7]. The study of diffusion of *Retinalamin* in sclera is important for estimation of a drug dose delivered into eye tissues at subconjuctival injection, since this method provides the highest concentration of drug in target area [8]. However, despite numerous investigations related to drug delivery in sclera [8–13] the problem of estimation of drug penetration rate in the tissue has not been studied in detail.

Diffusion coefficient of immersion fluid in fibrous tissue can be estimated by the method based on the measurements of temporal changes of optical properties of a tissue (transmittance or reflectance) caused by dynamic refractive index matching [14]. It can be used both for *in vitro* and *in vivo* measurements [11,13–16]. It is well known that sclera mainly consists of collagen fibers packed in lamellar bundles that are immersed in an amorphous base substance [17,18]. Inhomogeneities in the structure and difference between refractive indices of collagen fibers ($n_{col} = 1.411$) [19] and interstitial fluid ($n_{int} = 1.345$) [17] give a high scattering of sclera in the normal state [14,20]. Since refractive index of aqueous *Retinalamin* is higher than that of the interstitial fluid of sclera it has to induce optical clearing of the tissue.

In this paper we present the results of *in vitro* experiments on the human sclera optical properties control by administration of *Retinalamin*. The goal of the study is investigation of scleral permeability as the most significant tissue barrier at the topical application of the agent.

2. Materials and methods

2.1. Materials

In the study, 10 samples of human sclera obtained from three subjects were used. The samples were obtained from enucleated

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doi:10.1016/j.optlaseng.2008.07.006

eyes during elective surgery—enucleation of blind eye forming functioning stump for artificial eye. Age of the subjects was 38–45, all of them were males. The reasons for the enucleation were the followings: congenital absolute glaucoma and preathrophy owing to penetrating wound. Time interval from the enucleation to spectroscopic measurements did not exceed 24 h. After the enucleation all samples were kept in isotonic saline (0.9% NaCl) at the temperature 4–5 °C. All measurements were performed at room temperature of about 20 °C. Before the measurements, pigment layer (lamina fusca) was removed from the sclera samples. Thickness of the samples was measured by micrometer before the interaction with the studied solution and after that. Precision of the measurements was $\pm 50\,\mu m$. Obtained values were averaged.

Aqueous solution of *Retinalamin* (Geropharm Ltd., Russia) was used as an immersion fluid. Concentration of *Retinalamin* was 25 mg/ml. Refractive index was measured by Abbe refractometer as 1.346 at $\lambda = 589$ nm. Refractive index of the scleral interstitial fluid (after keeping in saline for 24 h) could be considered as matched to refractive index of weak saline, i.e. 1.332, which is close to that of water.

2.2. Experimental setup

Estimation of diffusion coefficient of the studied solution was made on the basis of analysis of reflectance dynamics of the tissue samples. The measurements of the scleral reflectance were performed in the spectral range 450–900 nm using a commercially available optical multichannel spectrometer LESA-5 (BioSpec, Russia). The scheme of the experimental setup is shown in Fig. 1.

As a light source a halogen lamp with filtering of the radiation in the spectral range 450–900 nm was used in the measurements. Light was delivered to the sample and collected from the tissue using a fiber-optical probe. The fiber-optical probe consisted of seven optical fibers. All fibers had 200 μm core diameter and a numerical aperture of 0.22. The central fiber S (see, Fig. 1) delivered the incident light to the tissue surface and six fibers D (the fibers were placed around the central fiber) collected the reflected light. Distance between centers of the delivering and receiving fibers was 290 μm. As a reference, a white slab $BaSO_4$ with a smooth surface was used. For the spectrometric measurements each sample was fixed on a cuvette with solution of *Retinalamin*. The reflectance spectrum of the sample was measured before administration of the agent solution and every 30 s during the first 10–15 min of the interaction. Then the measurements were carried out every 1–2 min up to the moment when the changes of the spectrum were negligible.

2.3. Method for estimation of diffusion coefficient

Transport of a drug within tissues can be described in the framework of free-diffusion model. The model of free diffusion can be used on the basis of difference of size of *Retinalamin* molecule (hydrodynamic radius of molecules with similar molecular weight (MW) is 2–3 nm [9]) and sizes of both collagen fibrils and interfibrillar space in sclera. According to [14], mean diameter of collagen fibrils is about 100 nm, and mean fibril displacement is ~285 nm. Thus, distance between the collagen fibrils is enough for free pass of the *Retinalamin* molecules between the fibrils. It is clear that *Retinalamin* diffusion rate in interstitial fluid of sclera should be different from the mean diffusion rate in the whole sclera due to porosity of the tissue and tortuosity of the diffusion pathway. However, the goal of this study is not investigations of diffusion of *Retinalamin* in the interstitial fluid of the sclera, but it is estimation of mean drug penetration rate (i.e. diffusion coefficient) through sclera, as a comprehensive whole, into inner eye space because it is important for ophthalmologists to estimate a drug dose delivered into retina. In our model, sclera is presented as a continuous medium with "effective" characteristics and, so, *Retinalamin* diffusion coefficient is measured for the medium. The use of free-diffusion model was well tested for molecules with large molecular weighs by different authors [9,10,21,22]. For example, Papadopoulos et al. [21],

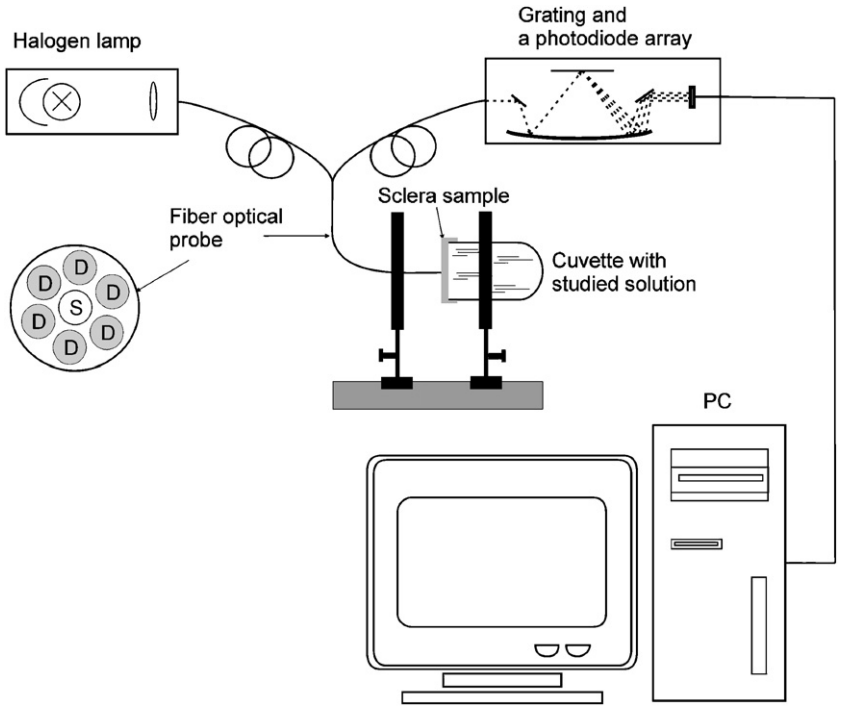

Fig. 1. Experimental setup for measurements of scleral reflectance spectra. S and D mean source and detector fibers, respectively.

analyzing the axial spread of the microinjected proteins in the muscle fibers, have shown that diffusion of large molecules (for example, myoglobin) is well described by the model. In the papers [9,10,22] the model is also used for describing diffusion of molecules with MW 0.023–150 kDa. As an alternative method to obtain the diffusivity the Monte Carlo (MC) simulation can be used, but it requires a lot of computing time.

We assume that the following approximations are valid for the transport process: (1) only concentration diffusion takes place; i.e., the flux of drug into tissue, at a certain point within the tissue sample, is proportional to the drug concentration at this point; (2) the diffusion coefficient is constant over the entire sample volume.

Geometrically the tissue sample is presented as a plane-parallel slab with a finite thickness. Since lateral dimensions of the experimental samples were much bigger than their thickness and lateral sides were fixed (not connected with the solution), the one-dimensional diffusion problem has been solved. The one-dimensional diffusion equation of the drug transport has the form (Fick's second law)

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}, \tag{1}$$

where $C(x,t)$ is the concentration of drug, g/ml; D the diffusion coefficient, cm^2/s; t the time, s; and x the spatial coordinate, cm.

We also suppose that penetration of drug into tissue does not change the concentration of the drug in the external volume. Besides, due to geometry of the measurements, penetration of *Retinalamin* into the sclera sample takes place from external surface of the sample only. The corresponding boundary conditions are

$$C(0,t) = C_0 \quad \text{and} \quad \frac{\partial C(l,t)}{\partial x} = 0, \tag{2}$$

where C_0 is the drug concentration in the external solution, g/ml, and l the sclera sample thickness, cm.

The initial condition corresponds to the absence of drug inside sclera before the measurements:

$$C(x,0) = 0 \tag{3}$$

for all inner points of the sample.

Solution of Eq. (1) for a slab with thickness l at the moment t with boundary (Eq. (2)) and initial (Eq. (3)) conditions has the form [23]

$$C(t) = C_0\left(1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-(2i+1)^2 t\frac{\pi^2}{4}D\Big/l^2\right)\right), \tag{4}$$

where $C(t)$ is the volume-averaged concentration of agent within the sclera sample.

The time dependence of refractive index of the interstitial fluid can be derived using the law of Gladstone and Dale, which states that the resulting value represents an average of the refractive indices of the components related to their volume fractions [19]. Such dependence is defined as

$$n_l(t) = (1 - C(t))n_{base} + C(t)n_{osm}, \tag{5}$$

where n_{base} is the refractive index of the tissue interstitial fluid at the initial moment (as was mentioned above the refractive index of the interstitial fluid is equal to the refractive index of water), and n_{osm} is the refractive index of the *Retinalamin* solution. The wavelength dependence of water has been presented by Kohl et al. [24] as

$$n_w(\lambda) = 1.3199 + \frac{6.878\times10^3}{\lambda^2} - \frac{1.132\times10^9}{\lambda^4} + \frac{1.11\times10^{14}}{\lambda^6}. \tag{6}$$

The optical model of tissue can be presented as a slab with a thickness l containing scatterers (collagen fibrils)—thin dielectric cylinders with an average diameter of 100 nm, which are considerably smaller than their lengths. Refractive index of these cylinders is 1.411 [19]. These cylinders are located in planes that are parallel to the sample surfaces, but within each plane their orientations are random. These simplifications reduce the difficulties in the description of light scattering by the tissue considerably.

As a first approximation, we assume that during the interaction between the tissue and the immersion liquid the size of the scatterers does not change. In this case, all changes in the tissue scattering are connected with the changes of the refractive index of the interstitial fluid described by Eq. (5). The increase of the refractive index of the interstitial fluid decreases the relative refractive index of the scattering particles and, consequently, decreases the scattering coefficient.

In the visible spectral range, the absorption coefficient of a tissue is much lesser than the scattering coefficient, except in blood absorption bands. Since *Retinalamin* does not have strong absorption bands within the wavelength range investigated, the changes of scleral tissue reflectance can be described only by the behavior of the scattering coefficient.

For calculation of the scleral reflectance we used MC algorithm developed by L. Wang et al. and presented in Ref. [25]. We have modified the subroutine of input incident photons and the subroutine of registration of photons backscattered by the tissue, taking into account the geometry of the fiber-optical probe used in the measurements (see, Section 2.2). The stochastic numerical MC method is widely used to model optical radiation propagation in complex randomly inhomogeneous highly scattering and absorbing media such as biological tissues [13,26–28]. Basic MC modeling of an individual photon packet trajectory consists of a sequence of elementary simulations [25]: photon path length generation, scattering and absorption events, and reflection or/and refraction on the medium boundaries. The initial and final states of the photons are entirely determined by the source and detector geometry. For simplicity, the incident light is assumed to be a pencil beam and the photons packets are launched normal to the tissue surface. The specular reflection from air–tissue surface is taken into account in the simulations. At the scattering site a new photon packet direction is determined according to the Henyey–Greenstein scattering phase function

$$f_{HG}(\theta) = \frac{1}{4\pi}\frac{1-g^2}{(1+g^2-2g\cos\theta)^{3/2}},$$

where θ is the polar scattering angle. The distribution over the azimuthal scattering angle was assumed as uniform. MC technique requires values of absorption and scattering coefficients, anisotropy factor, thickness, and refractive index of sclera. The required data and optical parameters have been presented earlier [29].

Both scattering coefficient and anisotropy factor have been calculated on the basis of Mie theory [30]. For non-interacting particles the time-dependent scattering coefficient $\mu_s(t)$ of a tissue is defined by the following equation:

$$\mu_s(t) = N\sigma_s(t), \tag{7}$$

where N is the number of the scattering particles (fibrils) per unit area and $\sigma_s(t)$ the time-dependent cross-section of scattering. The number of scattering particles per unit area can be estimated as $N = \phi/(\pi a^2)$ [31], where ϕ is the volume fraction of tissue scatterers and a is their radii. For typical fibrous tissues, such as sclera, ϕ is usually equal to 0.2–0.3 [26].

To take into account interparticle correlation effects, which are important for tissues with densely packed scattering particles, the

scattering cross-section has to be corrected by the packing factor of the scattering particles, $(1-\phi)^{p+1}/(1+\phi(p-1))^{p-1}$ [32], where p ($=2$ for rod-shaped particles) is a packing dimension that describes the rate at which the empty space between scatterers diminishes as the total number density increases.

In accordance with Mie theory [30], if incident light is non-polarized, scattering properties of cylindrical particles (the collagen fibrils and fibers) can be described by the following set of relations:

$$\sigma_s = 2aQ_s = 2a\frac{Q_{sI}+Q_{sII}}{2}, \tag{8}$$

where a is a radius of the cylinder and Q_s is an efficiency factor of scattering.

$$Q_{sI} = \frac{2}{x}\left[|b_{0I}|^2 + 2\sum_{n=1}^{\infty}(|b_{nI}|^2+|a_{nI}|^2)\right],$$
$$Q_{sII} = \frac{2}{x}\left[|a_{0II}|^2 + 2\sum_{n=1}^{\infty}(|a_{nII}|^2+|b_{nII}|^2)\right],$$

$$a_{nI} = \frac{C_nV_n - B_nD_n}{W_nV_n + iD_n^2}, \quad b_{nI} = \frac{W_nB_n + iD_nC_n}{W_nV_n + iD_n^2},$$

$$a_{nII} = -\frac{A_nV_n - iC_nD_n}{W_nV_n + iD_n^2}, \quad b_{nII} = -i\frac{C_nW_n + A_nD_n}{W_nV_n + iD_n^2},$$

$$A_n = i\xi[\xi J_n'(\eta)J_n(\xi) - \eta J_n(\eta)J_n'(\xi)],$$
$$D_n = n\cos\zeta\eta J_n(\eta)H_n^{(1)}(\xi)\left(\frac{\xi^2}{\eta^2}-1\right),$$

$$B_n = \xi[m^2\xi J_n'(\eta)J_n(\xi) - \eta J_n(\eta)J_n'(\xi)],$$
$$C_n = n\cos\zeta\eta J_n(\eta)J_n(\xi)\left(\frac{\xi^2}{\eta^2}-1\right),$$

$$V_n = \xi[m^2\xi J_n'(\eta)H_n^{(1)}(\xi) - \eta J_n(\eta)H_n'^{(1)}(\xi)],$$
$$W_n = i\xi[\eta J_n(\eta)H_n'^{(1)}(\xi) - \xi J_n'(\eta)H_n^{(1)}(\xi)],$$

$$\xi = x\sin(\zeta), \quad \eta = x\sqrt{m^2-\cos^2(\zeta)}.$$

Here ζ is the angle between the direction of the incident field and the axis of cylinder. In this paper we consider case where the wave vector of the incident field is directed perpendicular to the axis of cylinder ($\zeta = 90^\circ$); then the coefficients a_{nI} and b_{nII} become zero, and

$$b_{nI}(\zeta=90^\circ) = b_n = \frac{J_n(mx)J_n'(x) - mJ_n'(mx)J_n(x)}{J_n(mx)H_n'^{(1)}(x) - mJ_n'(mx)H_n^{(1)}(x)},$$

$$a_{nII}(\zeta=90^\circ) = a_n = \frac{mJ_n(mx)J_n'(x) - J_n'(mx)J_n(x)}{mJ_n(mx)H_n'^{(1)}(x) - J_n'(mx)H_n^{(1)}(x)}.$$

Here $J_n(\rho)$ is the Bessel function of the first kind of order n, $H_n^{(1)}(\rho)$ the Bessel function of the third kind of order n, $m = n_s/n_I$ the ratio of refractive indices of the particle (n_s) and surrounding medium (n_I), and $x = 2\pi a n_I/\lambda$ the size parameter, where λ represents wavelength in the surrounding medium.

Anisotropy factor of light scattering g (average cosine of scattering angle) for the case of an infinite cylinder illuminated by non-polarized light is defined by the following relation [30]:

$$g = \langle\cos\theta\rangle = \frac{\int_0^\pi \frac{T_{11}}{T_{11norm}}\cos(\theta)\sin(\theta)\,d\theta}{\int_0^\pi \frac{T_{11}}{T_{11norm}}\sin(\theta)\,d\theta}, \tag{9}$$

$$T_{11} = \frac{|T_1|^2+|T_2|^2}{2},$$
$$T_{11norm} = \frac{|b_{0I}+2b_{nI}\cos\theta|^2 + |a_{0II}+2a_{nII}\cos\theta|^2}{2},$$
$$T_1 = b_{0I} + 2\sum_{n=1}^{\infty} b_{nI}\cos(n\theta),$$
$$T_2 = a_{0II} + 2\sum_{n=1}^{\infty} a_{nII}\cos(n\theta),$$

where T_1, T_2 are the components of the forward scattering amplitude matrix; T_{11} is the component of the scattering matrix.

The set of equations describing *Retinalamin* concentration dependence on time represents the direct problem. The calculation of the diffusion coefficient of *Retinalamin* in the scleral tissue was carried out on the basis of measurement of the temporal evolution of optical reflectance. The solution of the inverse problem was obtained by minimization of the target function:

$$F(D) = \sum_{i=1}^{N_t}(R(D,t_i) - R^*(t_i))^2,$$

where $R(D,t)$ and $R^*(t)$ are the calculated and experimental values of time-dependent reflectance, respectively, and N_t is the number of time points obtained at registration of the temporal dynamics of reflectance. To minimize the target function the Levenberg–Marquardt nonlinear least-squares-fitting algorithm described in detail by Press et al. [33] was used. Iteration procedures were repeated until experimental and calculated data were matched. Calculations were performed for four wavelengths, and the obtained values of the diffusion coefficient have been averaged.

3. Results and discussion

Figs. 2 and 3 present reflectance spectra and dynamics of the reflectance at selected wavelengths, respectively. They characterize the change in the optical properties of the scleral tissue during interaction with *Retinalamin* solution.

Influence of water penetration on the change of scleral reflectance was studied by analogical method. Fig. 4 presents dynamics of human scleral optical reflectance measured at different wavelengths during interaction of the sample with saline.

As evidently follows from Fig. 2, at the initial moment the sclera sample is only slightly transparent for optical radiation.

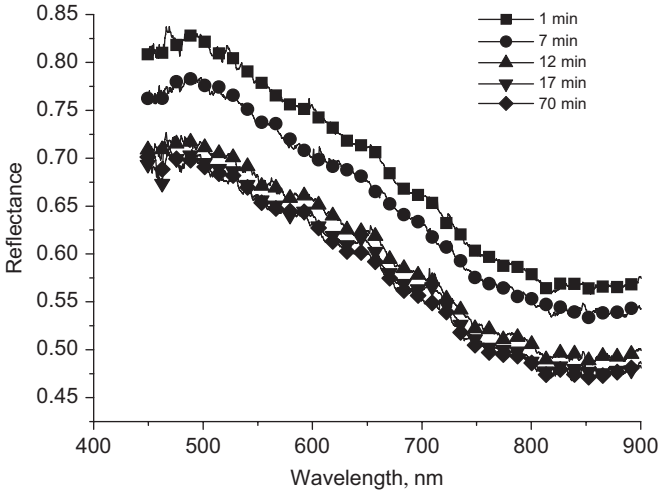

Fig. 2. Reflectance spectra of human eye sclera measured in different moments of interaction of the tissue with *Retinalamin* solution.

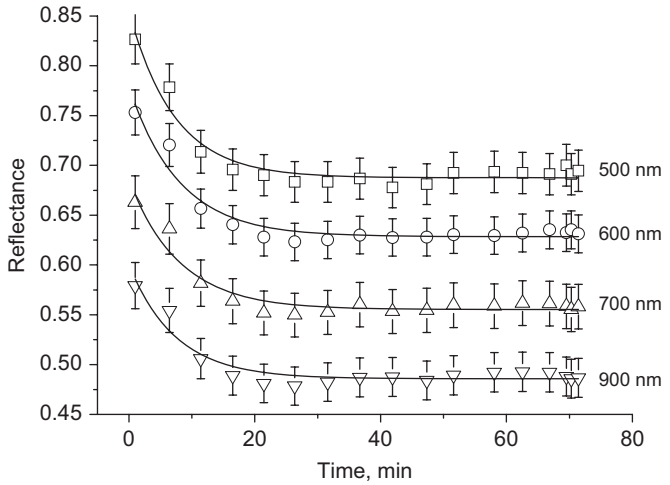

Fig. 3. Reflectance dynamics of human eye sclera measured at different wavelengths during interaction of the tissue with *Retinalamin* solution. Symbols present experimental data, solid lines correspond to approximated dependence, and the vertical lines are the standard deviation values.

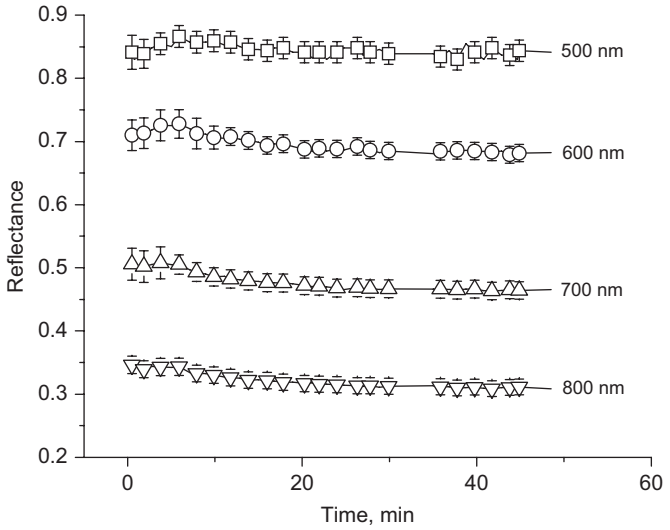

Fig. 4. Reflectance dynamics of human eye sclera measured at different wavelengths during interaction of the tissue with saline. Symbols present experimental data, and the vertical lines are the standard deviation values.

The form of reflectance spectra is determined by spectral dependence of sclera scattering coefficient. Since absorption coefficients of scleral collagen as well as water and proteins of interstitial matrix are insignificant in the spectral range studied, we can neglect light absorption in sclera [26].

The light-scattering properties of sclera are determined by both its structure and the relation of the refractive indices of the scatterers (mainly collagen fibers) and the scleral interstitial fluid that fills the space between the collagen fibers. On contact of fibrous tissue with immersion agent a few main mechanisms of clearing take place [13,14,34–36]: (1) partial dehydration of the tissue, (2) partial replacement of the interstitial fluid by the immersion substance, and (3) structural modification or dissociation of collagen. Both the first and the second processes mostly cause matching of the refractive indices of the tissue scatterers (mainly collagen fibers) and the cytoplasm and/or interstitial fluid. Tissue dehydration and structural modification lead to tissue shrinkage, i.e., to the near-order spatial correlation of scatterers and, as a result, the increased constructive interference of the elementary scattered fields in the forward direction and destructive interference in perpendicular direction of the incident light, that may significantly increase tissue transmittance even at some refractive index mismatch [34]. As a result, light scattering decreases and reflectance decreases (see, Fig. 3). It is well seen in Fig. 4 that water diffusion into the sclera sample practically did not change the scleral reflectance in the whole of the studied spectral range. Thus, it can be concluded that the changes in the scleral reflectance spectra observed in Figs. 2 and 3 were caused by the influence of *Retinalamin*.

It is important that the use of such high-polymer substances such as *Retinalamin* as an immersion agent leads to osmotic dehydration of tissue. The thickness of the samples decreases to about 20%; for example, before measurements the thickness of the sclera sample was 0.50 ± 0.08 mm, after that it was 0.38 ± 0.05 mm. Dehydration of the studied samples on the one hand leads to the increase of transmission due to the decrease of tissue thickness. On the other hand, it causes significant increase of scatterer volume fraction in tissue; which induces a rise of scattering coefficient and partly compensates for the immersion effect.

Fig. 3 demonstrates a good fit between the experimental data (points) and the approximating curves (solid lines) generated within the framework of the proposed model. The difference between experimental and calculated data can be explained partially by inaccuracy of the measurements and simplicity of the used model, since the diffusion coefficient can change a little during penetration of the clearing agent into the tissue, which is inhomogeneous through its volume.

The data on the optical reflectance dynamics have allowed us to estimate the diffusion coefficient of the agent in the sclera. Calculations were made for four wavelengths (500, 600, 700, and 800 nm) and averaged. The obtained value of the diffusion coefficient of *Retinalamin* is $(1.82 \pm 0.14) \times 10^{-6}$ cm^2/s.

Previous *in vitro* experiments have demonstrated that sclera is permeable to molecules as large as 150 kDa [9,22]. Diffusion of proteins with high MW in samples of fibrous tissues measured by fluorescent methods was presented in papers [9,21,22]. The value of *Retinalamin* diffusion coefficient is in good correspondence with the data obtained for other agents with similar molecular masses. Scleral permeability to fluorescein isothiocyanate—dextrans ranging in molecular mass from 4 to 150 kDa at 25 °C was determined by Ambati et al. [9]. The results have allowed us to estimate diffusion coefficient of dextrans with molecular masses 4.4 and 19.6 kDa as approximately 1.01×10^{-6} and 0.3×10^{-6} cm^2/s, respectively (permeability coefficient $P = D/l$, where l is the mean thickness of sclera sample, which is ~0.5 mm). From the data presented by Olsen et al. [22] we have estimated diffusion coefficient of dextran (10 kDa) as $\sim 0.4 \times 10^{-6}$ cm^2/s. The diffusion coefficients of cytochrome (12.4 kDa) and myoglobin (17 kDa) in muscle fibers measured at 22 °C amount to approximately 0.16×10^{-6} and 0.19×10^{-6} cm^2/s, respectively [21]. The differences between the diffusivities obtained in this paper and presented by the other authors can be explained by the differences in structure and properties of the investigated agents and tissues. The differences can be due to the various experimental and calculation methods.

From experimental data presented in Fig. 3 we can estimate diffusion length as a quantitative measure of how far *Retinalamin* solution has propagated in the chosen direction during the characteristic diffusion time. The value of characteristic diffusion time is 7.6 ± 0.3 min. Thus, the value of diffusion length is about 0.2 mm.

4. Conclusion

The results of the experiments have shown that penetration of *Retinalamin* into scleral tissue leads to a decrease of scleral reflection due to optical immersion. Analysis of reflectance dynamics of the sclera samples allowed us to estimate the diffusion coefficient of *Retinalamin* in sclera as $(1.82 \pm 0.14) \times 10^{-6}$ cm^2/s. The obtained result is in good correspondence with data presented in literature for other agents with similar molecular masses. The result is important for treatment of partial optic atrophy observed in primary open-angle glaucoma and other eye diseases.

Acknowledgements

The research described in this publication has been made possible, in part, by Grant REC-006 of US Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF), Grant of the Russian Federal Agency of Education Russian Federation 1.4.06, Grant no. 06-02-16740 of RFBR, and IREC "Photonics" of Innovative Universities Program of RF.

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